

**REMARKS**

The claims 1, 4, and 15-18 have been amended. Claim 5 has been cancelled. The sequence of claim 5 has been added to claim 4 to correct a mis-numbering of the claims. SEQ ID NOS have been added to claims 1, 4, 15-17 as well as to the specification (p. 15; Tables 2, 3, 3b, 4, and 5; Figures 1 and 4) and the abstract. These amendments have been made to conform to 37 CFR 1.821(d). Paper and electronic copies of the corrected Sequence Listing are enclosed herewith. Several grammatical errors have been corrected on pages 3 and 4 of the specification and in the bibliography. Clean versions of the specification, bibliography and figures are enclosed herewith. A statement regarding the U.S. government's rights to this invention has been added. No new matter has been entered with these amendments.

**Conclusion**

Applicants' attorney respectfully solicits a Notice of Allowance in this application. The Commissioner is authorized to charge any additionally required fees to deposit account 14-0460. Should the Examiner have any questions, comments, or suggestions that would expedite the prosecution of the present case to allowance, Applicants' undersigned representative earnestly requests a telephone call at (303) 384-7575.

Respectfully Submitted,

Date: \_\_\_\_\_

3/5/04

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CLEAN VERSION OF THE SPECIFICATION

The following replacement paragraphs are presented:

Following the title, please insert the following paragraph:

The United States Government has rights in this invention under contract number DE-AC36-99G0-10337 between the United States Department of Energy and the National Renewable Energy Laboratory, a division of the Midwest Research Institute.

On page 3, lines 16-27:

A method for reducing the glycosylation of an expressed *Trichoderma reesei* CBHI protein by site-directed mutagenesis ("SDM") is disclosed. The method includes replacing an N-glycosylation site amino acid residue, such as asparagines 45, 270, and/or 384 (referenced herein as CBHI-N45A, CBHI-N270A and CBHI-N384A, respectively), with a non-glycosyl accepting amino acid residue, such as is alanine. Various mutagenesis kits for SDM are available to those skilled in the art and the methods for SDM are well known. The description below discloses a procedure for making and using CBHI variants: CBHI-N45A; CBHI-N270A; and CBHI-N384A. The examples below demonstrate the expression of active CBHI in the heterologous fungus *Aspergillus awaniori*.

Variants of CBH I embodiments include mutations that provide for improved end product inhibition and for thermal tolerance.

On page 3, lines 31-33:

Figure 1. Coding sequence for the *cbhI* gene (SEQ ID NO: 4). Small case letters represent the signal sequence, large case letters the catalytic domain, bolded italics the linker region, and large case underlined the cellulose-binding domain.

On page 4, lines 7-8:

Figure 4. Coding sequence, SEQ ID NO: 19, for the linker region of the *cbhI* gene, SEQ ID NO: 4, showing additional proline nucleotides that effect conformation of the linker region in the protein structure.

On page 4, lines 12-24:

Acquisition of the gene was done by either cDNA cloning or by PCR of the gene from genomic DNA. CBH I cDNA was isolated from a *T. reesei* strain RUT C-30 cDNA library constructed using a PCR-generated probe based on published CBH I gene sequences (Shoemaker, et al., 1983). The cDNA's were cloned (using the Zap Express cDNA kit from Stratagene; cat. #200403) into the XhoI and EcoRI site(s) of the supplied, pre-cut lambda arms. An XhoI site was added to the 3' end of the cDNA during cDNA synthesis, and sticky-ended RE linkers were added to both ends. After XhoI digestion, one end has an XhoI overhang, and the other (5' end) has an Eco RI overhang. The insert can be removed from this clone as an approximately 1.7 kb fragment using Sall or SpeI plus XhoI in a double digest. There are two Eco RI, one Barn HI, 3 SacI and one HindIII sites in the coding sequence of the cDNA itself. The plasmid corresponding to this clone was excised *in vivo* from the original lambda clone, and corresponds to pB210-5A. Thus, the cDNA is inserted in parallel with a Lac promoter in the pBK-CMV parent vector. Strain pB210-5A grows on LB + kanamycin (50 ug/mL).

On page 15, lines 16-22:

The present example demonstrates the utility of the present invention for providing a nucleic acid molecule having a nucleic acid sequence that has a sequence 5'-GGCGGAAACCCGCCTGGCACCACC-3' (SEQ ID NO: 3). The identified nucleic acid sequence presents a novel linker region nucleic acid sequence that differs from previously reported nucleic acid sequence by the addition of one (1) codon. The invention in some

aspects thus provides a nucleic acid molecule having a nucleic acid sequence that comprises a linker region of about 20 to 60 nucleotides identified here.

On pages 16-19:

Table 2. Proline mutations to improve thermal tolerance.

<b>Mutation</b>	<b>Native sequence and mutagenic oligonucleotide</b>
SEQ ID NO: 23 <i>S8P - native sense strand</i>	5'-GCACTCTCCAATCGGAGACTCACCCG-3'
SEQ ID NO: 24 Mutagenic sense strand	5'-GCACTCTCCAACCGGAGACTCACCCG-3'
SEQ ID NO: 25 Mutagenic anti-sense strand	5'-CGGGTGAGTCTCCGTTGGAGAGTGC-3'
SEQ ID NO: 26 <i>N27P - native sense strand</i>	5'-GGCACGTGCACTCAACAGACAGGCTCCG-3'
SEQ ID NO: 27 Mutagenic sense strand	5'-GGCACGTGCACTCCACAGACAGGCTCCG-3'
SEQ ID NO: 28 Mutagenic anti-sense strand	5'-CGGAGCCTGTCTGTGGAGTGACGTGCC-3'
SEQ ID NO: 29 <i>A43P - native sense strand</i>	5'-GGCGCTGGACTCACGCTACGAACAGCAGCACG-3'
SEQ ID NO: 30 Mutagenic sense strand	5'-GGCGCTGGACTCACCTACGAACAGCAGCACG-3'
SEQ ID NO: 31 Mutagenic anti-sense strand	5'-CGTGCTGCTGTTTCGTAGGGTGAGTCCAGCGCC-3'
SEQ ID NO: 32 <i>G75P - native sense strand</i>	5'-GCTGTCTGGACGGTGCCGCCTACGCG-3'
SEQ ID NO: 33 Mutagenic sense strand	5'-GCTGTCTGGACCTTGCCGCCTACGCG-3'
SEQ ID NO: 34 Mutagenic anti-sense strand	5'-CGCGTAGGCGGCAGGGTCCAGACAGC-3'
SEQ ID NO: 35 <i>G94P - native sense strand</i>	5'-GCCTCTCCATTGGCTTTGTCACCC-3'
SEQ ID NO: 36 Mutagenic sense strand	5'-GCCTCTCCATTCCCTTTGTCACCC-3'
SEQ ID NO: 37 Mutagenic anti-sense strand	5'-GGGTGACAAAGGGAATGGAGAGGC-3'

SEQ ID NO: 38 <i>E190P - native sense strand</i>	5'-GGCCAACGTTGAGGGCTGGGAGCC-3'
SEQ ID NO: 39 Mutagenic sense strand	5'-GGCCAACGTT <u>CC</u> GGGCTGGGAGCC-3'
SEQ ID NO: 40 Mutagenic anti-sense strand	5'-GGCTCCCAGCCC <u>GGA</u> ACGTTGGCC-3'
SEQ ID NO: 41 <i>S195P - native sense strand</i>	5'-GGCTGGGAGCCGTCATCCAACAACGCG-3'
SEQ ID NO: 42 Mutagenic sense strand	5'-GGCTGGGAGCCG <u>CC</u> ATCCAACAACGCG-3'
SEQ ID NO: 43 Mutagenic anti-sense strand	5'-CGCGTTGTTGGATG <u>G</u> CGGCTCCCAGCC-3'
SEQ ID NO: 44 <i>K287P - native sense strand</i>	5'-CGATACCACCAAGAAATTGACCGTTGTCACCC-3'
SEQ ID NO: 45 Mutagenic sense strand	5'-CGATACCACCAAG <u>CC</u> ATTGACCGTTGTCACCC-3'
SEQ ID NO: 46 Mutagenic anti-sense strand	5'-GGGTGACAACGGTCAATG <u>G</u> CTTGGTGGTATCG-3'
SEQ ID NO: 47 <i>A299P - native sense strand</i>	5'-CGAGACGTCGGGTGCCATCAACCGATAC-3'
SEQ ID NO: 48 Mutagenic sense strand	5'-CGAGACGTCGGGT <u>CC</u> CATCAACCGATAC-3'
SEQ ID NO: 49 Mutagenic anti-sense strand	5'-GTATCGGTTGATG <u>GG</u> ACCCGACGTCTCG-3'
SEQ ID NO: 50 <i>Q312P/N315P - native sense strand</i>	5'-GGCGTCACTTTCCAGCAGCCCAACGCCGAGCTTGG-3'
SEQ ID NO: 51 Mutagenic sense strand	5'-GGCGTCACTTTCC <u>CG</u> CAGCCCC <u>CC</u> CGCCGAGCTTGG-3'
SEQ ID NO: 52 Mutagenic anti-sense strand	5'-CCAAGCTCGGCG <u>GG</u> GGGCTG <u>CG</u> GGAAAGTGACGCC-3'
SEQ ID NO: 53 <i>G359P - native sense strand</i>	5'-GGCTACCTCTGGCGGCATGGTTCTGG-3'
SEQ ID NO: 54 Mutagenic sense strand	5'-GGCTACCTCT <u>CCC</u> GGCATGGTTCTGG-3'
SEQ ID NO: 55 Mutagenic anti-sense strand	5'-CCAGAACCATGCCG <u>GG</u> GAGAGGTAGCC-3'

SEQ ID NO: 56 <i>S398P/S401 P- native sense strand</i>	5'-GCGGAAGCTGCTCCACCAGCTCCGGTGTCCCTGC-3'
SEQ ID NO: 57 Mutagenic sense strand	5'-GCGGAAGCTGCC <u>CC</u> ACCAGC <u>CC</u> GGTGTCCCTGC-3'
SEQ ID NO: 58 Mutagenic anti-sense strand	5'-GCAGGGACACCGGGGCTGGTGGGCGCAGCTTCCGC-3'
SEQ ID NO: 59 <i>A414P - native sense strand</i>	5'-GTCTCCCAACGCCAAGGTCACC-3'
SEQ ID NO: 60 Mutagenic sense strand	5'-GTCTCCCAAC <u>CC</u> CAAGGTCACC-3'
SEQ ID NO: 61 Mutagenic anti-sense strand	5'-GGTGACCTTGGG <u>G</u> TTGGGAGAC-3'
SEQ ID NO: 62 <i>N431P/S433P - native sense strand</i>	5'-GGCAGCACCGGCAACCCTAGCGGCGGCAACCC-3'
SEQ ID NO: 63 Mutagenic sense strand	5'-GGCAGCACCGGCC <u>CC</u> CCCT <u>CC</u> GGGCGGCAACCC-3'
SEQ ID NO: 64 Mutagenic anti-sense strand	5'-GGGTTGCCGCCGGGAGGGGGGCCGGTGTGCC-3'

Table 3. Mutation to remove peptide strain.

Mutation site	Native sequence and mutagenic oligonucleotide
SEQ ID NO: 65 <i>S99G- native sense strand</i>	5'-GGCTTTGTCACCCAGTCTGCGCAGAAGAACGTTGGC-3'
SEQ ID NO: 66 Mutagenic sense strand	5'-GGCTTTGTCACCCAGGGTTCGCGAGAAGAACGTTGGC-3'
SEQ ID NO: 67 Mutagenic anti-sense strand	5'-GCCAACGTTCTTCTGCGCACCCTGGGTGACAAAGCC-3'

Table 3b. Y245G analogs to remove product inhibition.

Mutation site	Native sequence and mutagenic oligonucleotide
SEQ ID NO: 68 <i>R251A - native sense strand</i>	5'-CCGATAACAGATATGGCGGC-3'
SEQ ID NO: 69 Mutagenic sense strand	5'-CCGATAACGCCTATGGCGGC-3'
SEQ ID NO: 70 Mutagenic anti-sense strand	5'-GCCGCCATAGGGCGTTATCGG-3'
SEQ ID NO: 71 <i>R394A- native sense strand</i>	5'-CCCGGTGCCGTGCGCGGAAGCTGCTCCACC-3'

SEQ ID NO: 72 Mutagenic sense strand	5'-CCCGGTGCCGTG <u>GC</u> CGGAAGCTGCTCCACC-3'
SEQ ID NO: 73 Mutagenic anti-sense strand	5'-GGTGGAGCAGCTTCCG <u>GC</u> CACGGCACCGGG-3'
SEQ ID NO: 74 <i>F338A- native sense strand</i>	5'-GCTGAGGAGGCAGAAATTCGGCGGATCCTCTTTCTC-3'
SEQ ID NO: 75 Mutagenic sense strand	5'-GCTGAGGAGGCAGAAAG <u>GC</u> CGGCGGATCCTCTTTCTC-3'
SEQ ID NO: 76 Mutagenic anti-sense strand	5'-GAGAAAGAGGATCCGCCG <u>GC</u> TTCTGCCTCCTCAGC-3'
SEQ ID NO: 77 <i>R267A- native sense strand</i>	5'-GGAACCCATACCGCCTGGGCAACACCAGC-3'
SEQ ID NO: 78 Mutagenic sense strand	5'-GGAACCCATACG <u>GC</u> CCTGGGCAACACCAGC-3'
SEQ ID NO: 79 Mutagenic anti-sense strand	5'-GCTGGTGTGCCCAGG <u>GC</u> GTATGGGTTC-3'
SEQ ID NO: 80 <i>E385A- native sense strand</i>	5'-CCTACCCGACAAACGAGACCTCCTCCACACCCGG-3'
SEQ ID NO: 81 Mutagenic sense strand	5'-CCTACCCGACAAACG <u>GC</u> ACCTCCTCCACACCCGG-3'
SEQ ID NO: 82 Mutagenic anti-sense strand	5'-CCGGGTGTGGAGGAGGTG <u>GC</u> GTTTGTCGGGTAGG-3'

Table 4. N to A mutations to remove glycosylation.

Mutant	Native sequence and mutagenic oligonucleotide
SEQ ID NO: 20 <i>N45A - native sense strand</i>	5'-GGACTCACGCTACGAACAGCAGCACGAACTGC-3'
SEQ ID NO: 83 Mutagenic sense strand	5'-GGACTCACGCTACG <u>GC</u> CAGCAGCACGAACTGC-3'
SEQ ID NO: 84 Mutagenic anti-sense strand	5'-GCAGTTCGTGCTGCTG <u>GC</u> CGTAGCGTGAGTCC-3'
SEQ ID NO: 21 <i>N270A - native sense strand</i>	5'-CCCATACCGCCTGGGCAACACCAGCTTCTACGGCCC-3'
SEQ ID NO: 85 Mutagenic sense strand	5'-CCCATACCGCCTGGGCG <u>GC</u> CACCAGCTTCTACGGCCC-3'
SEQ ID NO: 86 Mutagenic anti-sense strand	5'-GGGCCGTAGAAGCTGGTG <u>GC</u> GCCCAGGCGGTATGGG-3'
SEQ ID NO: 22	5'-GGACTCCACCTACCCGACAAACGAGACCTCCTCCACACCCG-3'

<i>N384A - native sense strand</i>	
SEQ ID NO: 87	5'-GGACTCCACCTACCCGACAG <u>GCC</u> GAGACCTCCTCCACACCCG-3'
Mutagenic sense strand	
SEQ ID NO: 88	5'-CGGGTGTGGAGGAGGTCTCG <u>GCT</u> GTCGGGTAGGTGGAGTCC-
Mutagenic anti-sense strand	3'

Table 5. Helix capping mutations to improve thermal tolerance.

Mutant	Native sequence and mutagenic oligonucleotide
SEQ ID NO: 89 <i>E337R - native sense strand</i>	5'-GCTGAGGAGGCAGAATTCGGCGG-3'
SEQ ID NO: 90 Mutagenic sense strand	5'-GCTGAGGAGGCAC <u>GCT</u> TCGGCGG-3'
SEQ ID NO: 91 Mutagenic anti-sense strand	5'-CCGCCGAAG <u>GCG</u> TGCCTCCTCAGC-3'
SEQ ID NO: 92 <i>N327D - native sense strand</i>	5'-GGCAACGAGCTCAACGATGATTACTGC-3'
SEQ ID NO: 93 Mutagenic sense strand	5'-GGCAACGAGCTC <u>GAC</u> GATGATTACTGC-3'
SEQ ID NO: 94 Mutagenic anti-sense strand	5'-GCAGTAATCATCGT <u>CG</u> AGCTCGTTGCC-3'
SEQ ID NO: 95 <i>A405D - native sense strand</i>	5'-CCGGTGTCCCTGCTCAGGTCGAATCTCAGTCTCCC-3'
SEQ ID NO: 96 Mutagenic sense strand	5'-CCGGTGTCCCTG <u>AT</u> CAGGTCGAATCTCAGTCTCCC-3'
SEQ ID NO: 97 Mutagenic anti-sense strand	5'-GGGAGACTGAGATTCGACCTGA <u>T</u> CAGGGACACCGG-3'
SEQ ID NO: 98 <i>Q41OR - native sense strand</i>	5'-GCTCAGGTCGAATCTCAGTCTCCCAACGCC-3'
SEQ ID NO: 99 Mutagenic sense strand	5'-GCTCAGGTCGAATCTC <u>GCT</u> CTCCCAACGCC-3'
SEQ ID NO: 100 Mutagenic anti-sense strand	5'-GGCGTTGGGAGAG <u>GCG</u> GAGATTCGACCTGAGC-3'

SEQ ID NO: 101 <i>N64D - native sense strand</i>	5'-CCCTATGTCCTGACAACGAGACCTGCGCG-3'
SEQ ID NO: 102 Mutagenic sense strand	5'-CCCTATGTCCTGAC <u>G</u> ACGAGACCTGCGCG-3'
SEQ ID NO: 103 Mutagenic anti-sense strand	5'-CGCGCAGGTCTCGT <u>C</u> GTCAGGACATAGGG-3'
SEQ ID NO: 104 <i>N64D - native sense strand</i>	5'-GCTCGACCCTATGTCCTGACAACGAGACCTGCGCGAAGAACTGC-3'
SEQ ID NO: 105 Mutagenic sense strand	5'-GCTCGACCCTATGTCCTGAC <u>G</u> ACGAGACCTGCGCGAAGAACTGC-3'
SEQ ID NO: 106 Mutagenic anti-sense strand	5'-GCAGTTCTTCGCGCAGGTCTCGT <u>C</u> GTCAGGACATAGGGTCGAGC-3'

## CLEAN VERSION OF THE BIBLIOGRAPHY

Page 22, lines 39-41:

Expression and secretion of defined cutinase variants by *Aspergillus awamori*. VanGemerén, IA; Beijersbergen, A; van den Hondel, CAMJJ; Verrips, CT. **Appl. Environ. Microbiol.** August 1998 v64 i8 p2794-2799 (6).

Page 22, lines 47-49:

Influence of fermentation conditions and scale on the submerged fermentation of *Aspergillus awamori*. Cui, YQ; vanderLans, RGJM; Giuseppin, MLF; Luyben, KCAM. **Enzyme Microb. Technol.** July-August 1998 v23 i1-2 p157-167 (11).

Page 23, lines 1-3:

Intrinsic kinetic parameters of the pellet forming fungus *Aspergillus awamori*. Hellendoorn, L; Mulder, H; van den Heuvel, JC; Ottengraf, SPP. **Biotechnol. Bioeng.** June 5, 1998 v58 i5 p478-485 (8).

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The ER chaperone encoding bipA gene of black *Aspergilli* is induced by heat shock and unfolded proteins. vanGemerén, IA; Punt, PJ; DrintKuyvenhoven, A; Broekhuijsen, MP; vanthHoog, A; Beijersbergen, A; Verrips, CT; van den Hondel, CAMJJ. **Gene.** October 1, 1997 v198 i1-2 p43-52 (10).

Page 23, lines 43-45:

Glucoamylase gene fusions alleviate limitations for protein production in *Aspergillus awamori* at the transcriptional and (post)translational levels. Gouka, RJ; Punt, PJ; van den Hondel, CAMJJ. **Appl. Environ. Microbiol.** February 1997 v63 i2 p488-497 (10).

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Kinetics of mRNA and protein synthesis of genes controlled by the 1,4-beta-endoxylanase A promoter in controlled fermentations of *Aspergillus awamori*. Gouka, RJ; Stam, H; Fellingier, AJ; Muijsenberg, RJGT; vandeWijngaard, AJ; Punt, PJ; Musters, W; van den Hondel, CAMJJ. **Appl. Environ. Microbiol.** October 1996 v62 i10 p3646-3649 (4).

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An expression system based on the promoter region of the *Aspergillus awamori* 1,4-beta-endoxylanase A gene. Gouka, RJ; Hessing, JGM; Punt, PJ; Stam, H; Musters, W; van den Hondel, CAMJJ. **Appl. Microbiol. Biotechnol.** (1996) v46 i1 p28-35 (8).

Page 24, lines 10-12:

Analysis of heterologous protein production in defined recombinant *Aspergillus awamori* strains. Gouka, RJ; Punt, PJ; Hessing, JGM; van den Hondel, CAMJJ. **Appl. Environ. Microbiol.** (1996) v62 i6 p1951-1957 (7).

Figure 1. Coding sequence for the *cbh1* gene (SEQ ID NO: 4). Small case letters represent the signal sequence, large case letters the catalytic domain, bolded italics the linker region, and large case underlined the cellulose-binding domain.

atgtatcggaagttggccgtcatctcgcccttcttgccacagctcgtgctCAGTCGGCCTGCACTCTCCAATCGGAACTCAC  
CCGCCTCTGACATGGCAGAAATGCTCGTCTGGTGGCACGTGCACTCAACAGACAGGCTCCGTG  
GTCATCGACGCCAACTGGCGCTGGACTCACGCTACGAACAGCAGCACGAACTGCTACGATGG  
CAACACTTGGAGCTCGACCCATATGTCCTGACAACGAGACCTGCGCGAAGAACTGCTGTCTGGA  
CGGTGCCGCCTACGCGTCCACGTACGGAGTTACCACGAGCGGTAACAGCCTCTCCATTGGCTT  
TGTCACCCAGTCTGCGCAGAAGAACGTTGGCGCTCGCCTTTACCTTATGGCGAGCGACACGAC  
CTACCAGGAATTCACCCTGCTTGGCAACGAGTTCTCTTTCGATGTTGATGTTTCGCAGCTGCCG  
TGCGGCTTGAACGGAGCTCTCTACTTCGTGTCCATGGACGCGGATGGTGGCGTGAGCAAGTAT  
CCCACCAACACCGCTGGCGCCAAGTACGGCACGGGGTACTGTGACAGCCAGTGTCCTCCGCGA  
TCTGAAGTTCATCAATGGCCAGGCCAACGTTGAGGGCTGGGAGCCGTCATCCAACAACGCGA  
ACACGGGCATTGGAGGACACGGAAGCTGCTGCTCTGAGATGGATATCTGGGAGGCCAACTCC  
ATCTCCGAGGCTCTTACCCCCCACCCTTGCACGACTGTGCGCCAGGAGATCTGCGAGGGTGAT  
GGGTGCGGCGGAACTTACTCCGATAACAGATATGGCGGCACTTGCATCCCGATGGCTGCGA  
CTGGAACCCATACCGCCTGGGCAACACCAGCTTCTACGGCCCTGGCTCAAGCTTTACCCTCGA  
TACCACCAAGAAATTGACCGTTGTCACCCAGTTTCGAGACGTCGGGTGCCATCAACCGATACTA  
TGTCAGAAATGGCGTCACTTTCCAGCAGCCCAACGCCGAGCTTGGTAGTTACTCTGGCAACGA  
GCTCAACGATGATTACTGCACAGCTGAGGAGGCAGAATTCGGCGGATCCTCTTTCTCAGACAA  
GGGCGGCCTGACTCAGTTCAAGAAGGCTACCTCTGGCGGCATGGTTCTGGTCATGAGTCTGTG  
GGATGATTACTACGCCAACATGCTGTGGCTGGACTCCACCTACCCGACAAACGAGACCTCCTC  
CACACCCGGTGCCGTGCGCGGAAGCTGCTCCACCAGCTCCGGTGTCCCTGCTCAGGTGCAATC  
TCAGTCTCCCAACGCCAAGGTCACCTTCTCCAACATCAAGTTCGGACCCATTGGCAGCACCGG  
CAACCCTAGCGGCGGCAACCCTCCCGGCGGAAACCCGCCTGGCACCACCACCACCCGCCGCC  
***AGCCACTACCACTGGAAGCTCTCCCGGACCTACCCAGTCTCACTACGGCCAGTGCGGCGGTATT***  
***GGCTACAGCGGCCCCACGGTCTGCGCCAGCGGCACAACCTGCCAGGTCCTGAACCCTTACTAC***  
***TCTCAGTGCCTGTAAAGCTCC***

Figure 4. Coding sequence, SEQ ID NO: 19, for the linker region of the *cbh1* gene, SEQ ID NO: 4, showing additional proline nucleotides that effect conformation of the linker region in the protein structure.

